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(54) [Title of the Invention] Solubilizing agents for aculeacins and medicinal compositions therefrom.

(57) [ABSTRACT]
[Constitution] The present invention is aqueous compositions of aculeacins, or dried compositions thereof, wherein aculeacins and glycyrrhizin derivatives are contained. The invention is also compositions wherein sodium salicylate or sodium benzoate are added as solubilizing adjuvants, in order to increase the solubilities of the aculeacins in the above-mentioned compositions. Further, the invention is compositions wherein amino acids are added to the aforementioned compositions, in order to prevent turbidity when the above-mentioned compositions are dissolved in infusions.
[Effects] By employing glycyrrhizin derivatives as solubilizing agents for aculeacins, which are sparingly soluble in water, aqueous or dried compositions thereof may be solubilized very safely.

[SCOPE OF THE PATENT CLAIMS]

1. Solubilizing agents for aculeacins, wherein the active ingredients are glycyrrhizin derivatives.

2. Aqueous or dried compositions of aculeacins, wherein aculeacins and glycyrrhizin derivatives are contained.

3. Aqueous or dried compositions of aculeacins, wherein aculeacins, glycyrrhizin derivatives and sodium salicylate or sodium benzoate are contained.

4. Aqueous compositions of aculeacins or dried compositions thereof, wherein aculeacins, glycyrrhizin derivatives and amino acids are contained.

[DETAILED DESCRIPTION OF THE INVENTION]

[0001]

[Field of Industrial Utilization] The present invention relates to solubilizing agents for aculeacins and medicinal compositions therefrom.

[0002]

[Description of the Related Art] Aculeacins are produced by micro-organisms belonging to the genus *Aspergillus* and are known as antibiotics having antimycotic effects. (*Japanese Examined Patent Application 59-20350, Japanese Examined Patent Application 59-20351, Japanese Examined Patent*

Application 59-20352 and *Japanese Examined Patent Application 59-20353*). They are also expected to be prophylactic and therapeutic agents for *Pneumocystis carinii* pneumonia [*Japanese Unexamined Patent Application 2-288837; Tetrahedron Letters,* 4147-4150 (1976); *Helvetica Chimica Acta,* 62(4), 1252-1267 (1979)].

[0003] However, aculeacins are very sparingly soluble in water and it is difficult to solubilize them by uniformly dispersing them in an aqueous solution to the point where they are completely clear to the naked eye. For this reason, solubilizing agents such as alcohols, polyhydric alcohols and cholic acids are used for conventional solubilizing processes (*Japanese Unexamined Patent Application 2-288837*). In these processes, when dilution with physiological saline or the like is carried out after solubilization, there is turbidity, so that further addition of a non-ionic surfactant, such as HCO-60 or Tween 80 is needed. On the other hand, solubilization is difficult with a non-ionic surfactant alone. There are also problems from the aspect of safety with the addition of dissolution adjuvants such as the aforementioned surfactants.

[0004]

[Problems to be Solved by the Invention] Although the use of alcohols, polyhydric alcohols, cholic acids and non-ionic surfactants is indispensable for the solubilization of such

aculeacins, there are problems from the aspect of safety. An object of the present invention is therefore the provision of aculeacin compositions which are safe and stable.

[0005]

[The Means of Solving the Problems] As a result of diligent investigations into processes for solubilization, in order to solve the above problems, it has become possible to obtain compositions whereby aculeacins can be solubilized safely and highly efficiently, by the unexpected use of glycyrrhizin derivatives as solubilizing agents. It was further discovered that these compositions have excellent storage stabilities.

[0006] That is, the present invention provides aculeacin solubilizing agents wherein the active ingredients are glycyrrhizin derivatives. The present invention also provides aqueous compositions of aculeacins, or dried compositions thereof, wherein aculeacins and glycyrrhizin derivatives are contained. When the present inventors carried out investigations into dissolution adjuvants, in order to increase the solubilizing power for aculeacins, they discovered the surprising fact that the degree of solubility of aculeacins is markedly improved by the addition of sodium salicylate or sodium benzoate to the aforementioned compositions.

[0007] That is, the present invention provides aqueous compositions of aculeacins, or dried compositions thereof, wherein are contained aculeacins, glycyrrhizin derivatives and sodium salicylate or sodium benzoate. Methods generally considered for the administration of medicines are intravenous, intramuscular, intradermal and subcutaneous administration or the like; one of the most frequently used methods for the intravenous administration is to mix a medicine into an infusion, after which intravenous administration is carried out, but, when the present inventors carried out further investigations into methods for the administration of the above-mentioned aculeacin compositions, and an above-mentioned composition was mixed into an infusion, if the content of a solubilizing agent was markedly small, turbidity was seen after the mixing. As a result of various additional investigations for the purpose of preventing this turbidity, it was discovered that turbidity may be prevented by the addition to an above-mentioned composition of a specified amino acid, such as glutamic acid, aspartic acid or cysteine hydrochloride.

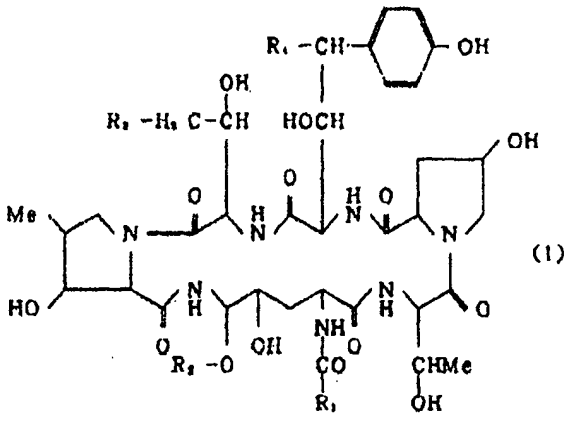
[0008] That is, the present invention provides aqueous compositions of aculeacins, or dried compositions thereof, wherein are contained aculeacins, glycyrrhizin derivatives and amino acids. The solubilization according to the invention may thus be described as dispersing aculeacins uniformly in aqueous solvents and making them into a state

whereby they are completely clear to the naked eye. An example of a suitable aqueous solvent which may be given is distilled water. To this may be added suitable salts, sugars and acids; examples of these which may be given are distilled water for injection, physiological saline, sugar solution and buffer solutions. The aforementioned aqueous solutions may also contain aqueous organic solvents, for example, ethanol, as long as they do not exhibit any toxicity. The aqueous compositions according to the present invention signify liquid compositions whereby aculeacins are solubilized by means of the above-mentioned aqueous solvents and these aqueous compositions may be produced as dry compositions by means of a suitable normal drying means.

[0009] The aculeacins which are the active ingredients according to the present invention are substances represented by the general formula (I):

[0010]

[Chemical Expression 1]



(1)

[0011] (in which formula R_1–O– denotes an organic acid residue, which may contain in the molecule, a long chain saturated or unsaturated fatty acid residue or benzene ring, pyridine ring, oxygen atom, iodine atom or nitrogen atom; R_2 denotes a hydrogen atom, a lower alkyl group, which may have a branched chain, a benzyl group, or an amino-lower alkyl group, wherein the amino group may be mono- or di-substituted with lower alkyl groups; R_3 denotes a hydrogen atom or $-CONH_2$ group; and R_4 denotes a hydrogen atom or a hydroxyl group).

[0012] Examples which may be given of the R_1 group in general formula (1) are:

[0013]

[Chemical Expression 2]

$-(CH_2)_n Me$ (n=10~20)、

$-(CH_2)_n CH=CH(CH_2)_7 Me$ (n=7, 9, 11)、

$-(CH_2)_8 CH=CH(CH_2)_7 Me$、

$-(CH_2)_7 CH=CH(CH_2)_8 Me$、

$-(CH_2)_8 CH=CH(CH_2)_{10} Me$、

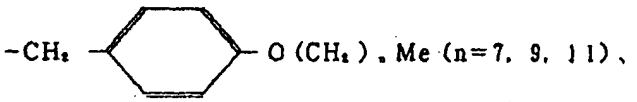
$-(CH_2)_7 CH=CHCH_2 CH=CH(CH_2)_4 Me$、

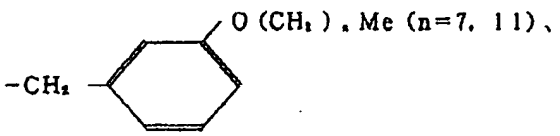
$-(CH_2)_7 CH=CHCH_2 CH=CHCH_2 CH=CHCH_2 Me$、

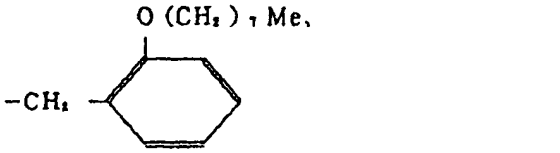
$-(CH_2)_8 CH(Me)-CH_2 CH(Me)-CH_2 Me$、

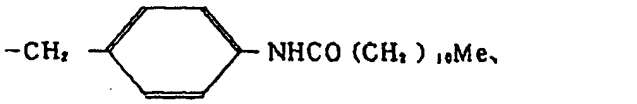
$-(CH_2)_{10}NHCO(CH_2)_6 Me$ (n=7, 10)、

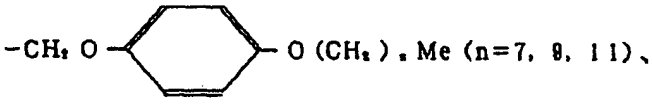
$-(CH_2)_6 NHCO(CH_2)_{10} Me$、





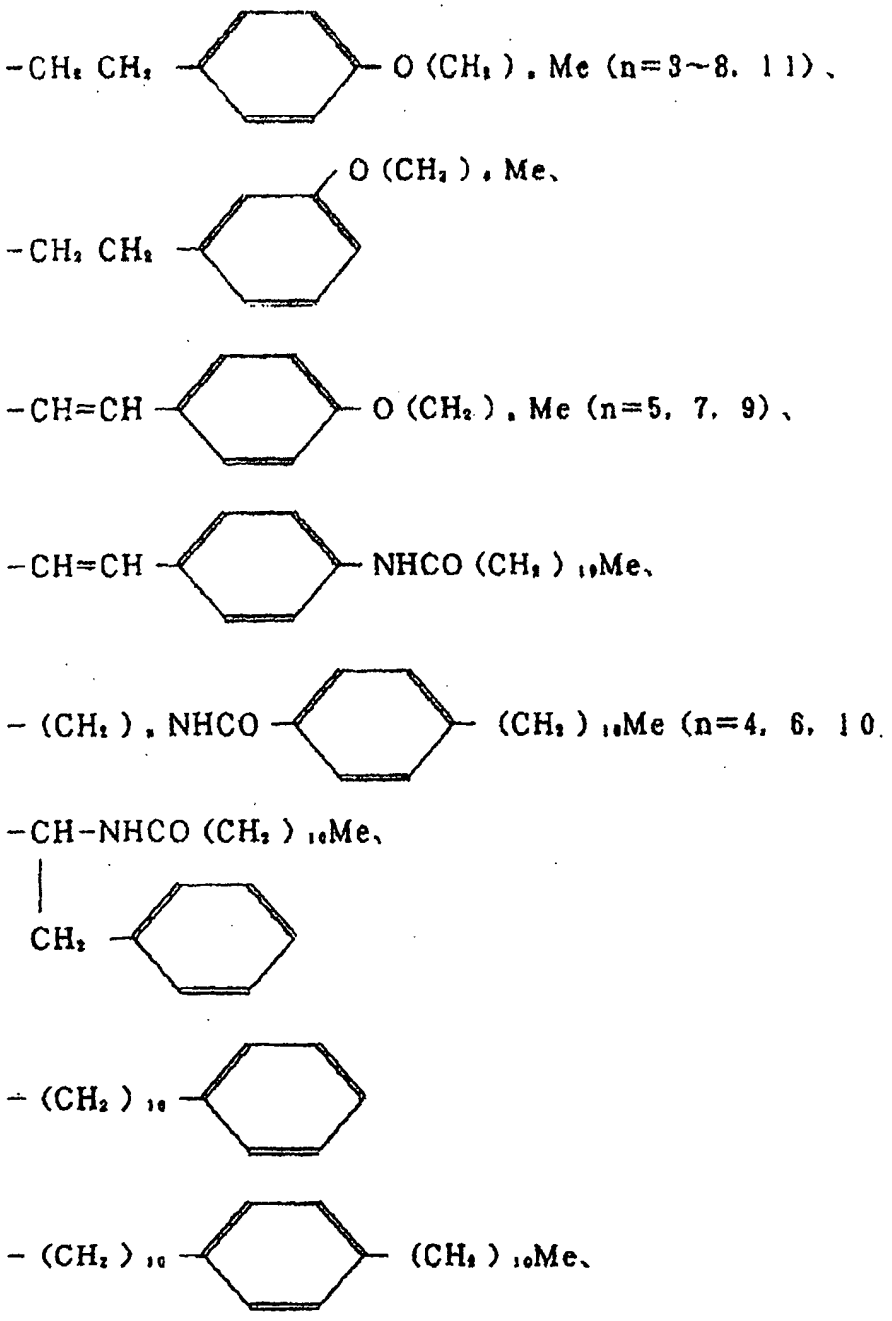






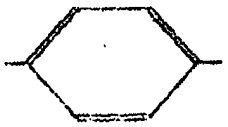
[0014]

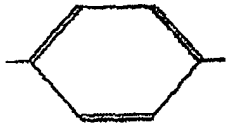
[Chemical Expression 3]

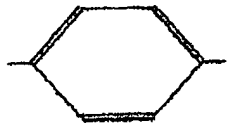


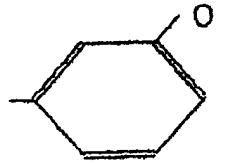
[0015]

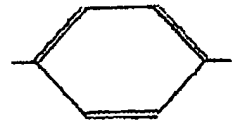
[Chemical Expression 4]

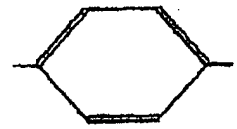


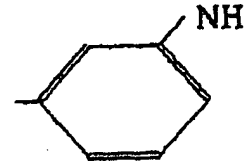






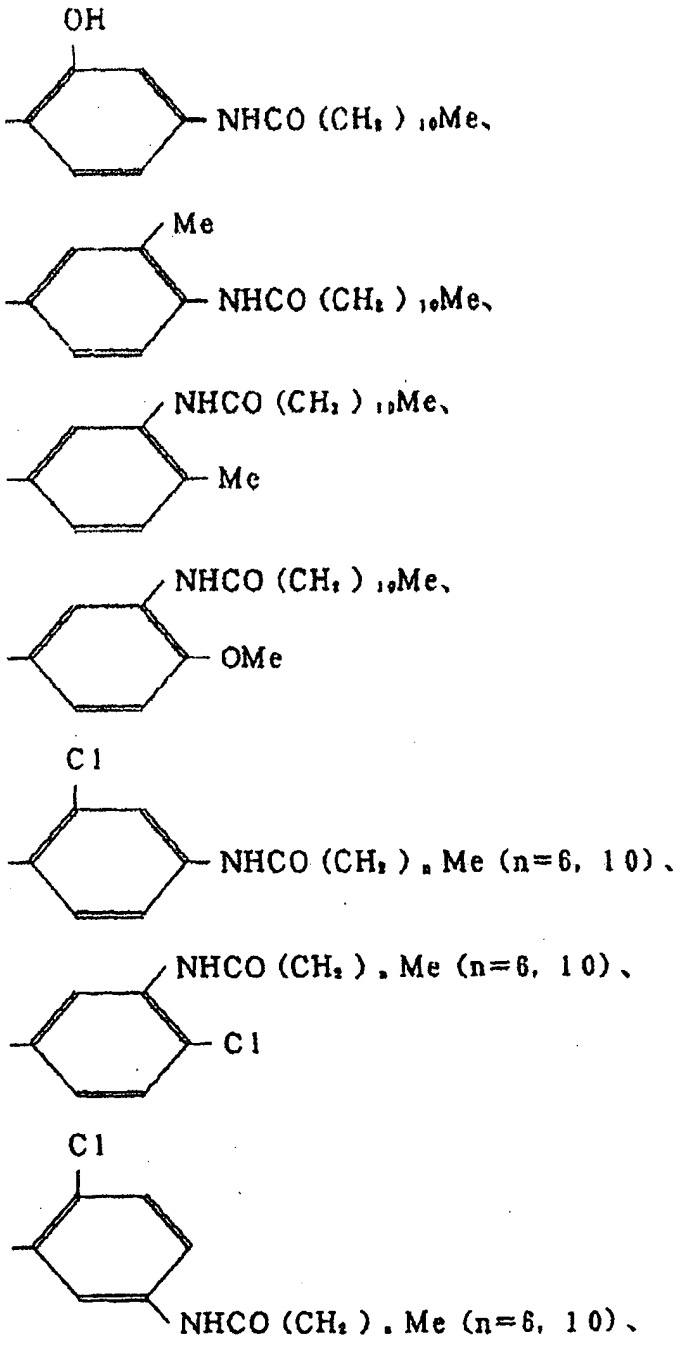






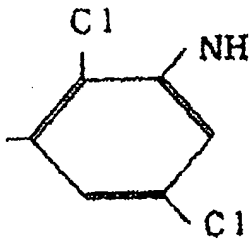
[0016]

[Chemical Expression 5]

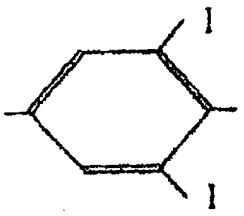


[0017]

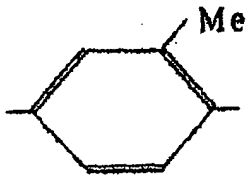
[Chemical Expression 6]



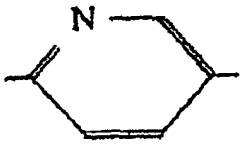
NHCO $(CH_2)_nMe$ (n=6, 8, 10, 12)、



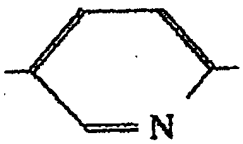
NHCO $(CH_2)_{10}Me$、



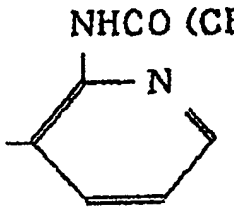
$CONH(CH_2)_{10}Me$、



O $(CH_2)_nMe$ (n=7, 11)、



O $(CH_2)_nMe$ (n=7, 11)、



[0018] Examples which may be given of the R_2 group are: a hydrogen atom, a straight chain or branched chain lower alkyl group of 1 to 6 carbons, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, *secondary* butyl, *tertiary* butyl, pentyl, hexyl, 3-methylbutyl, 2-ethylbutyl and 1-ethylbutyl; the benzyl group; amino-lower alkyl groups, such as 2-aminoethyl, 3-aminopropyl, 4-aminobutyl, 2-aminopropyl or 2-aminobutyl groups, or amino-lower alkyl groups, such as 2-aminoethyls and 3-aminopropyls, wherein the amino groups are mono- or di- substituted with lower alkyl groups, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl and secondary butyl groups.

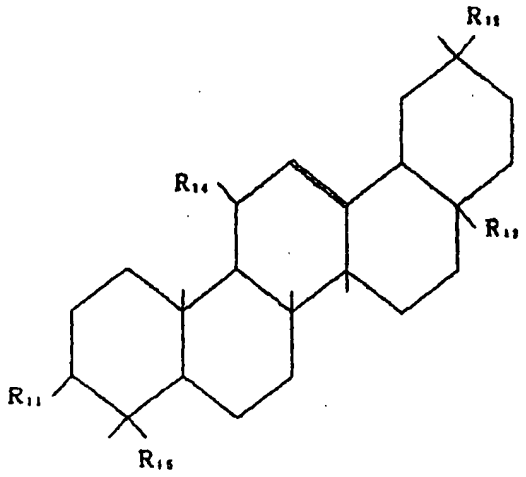
[0019] Examples of the R_3 group which may be given are a hydrogen atom or $-CONH_2$ and examples of the R_4 group which may be given are hydrogen or the hydroxyl group. In the above-mentioned general formula preferred aculeacin derivatives are those wherein (1), R_1-CO- is preferably a long chain saturated or unsaturated fatty acid residue, with, for example, 14 to 18 carbons (C_{14} to C_{18}); R_2 is preferably a hydrogen atom; R_3 is preferably a hydrogen atom; R_4 is preferably a hydrogen atom or a hydroxyl group; and more preferred examples which may be given are: aculeacin Aα, wherein R_1-CO- represents a myristic acid residue (C_{14}) and R_4 a hydroxyl group; aculeacin Aγ, wherein R_1-CO- represents a palmitic acid residue (C_{16}) and R_4 a hydroxyl group;

aculeacin Dα wherein R_1–CO– represents a myristic acid residue and R_4 represents a hydrogen atom; aculeacin Dγ, wherein R_1–CO– represents a palmitic acid residue and R_4 a hydrogen atom; echinocandin C, wherein R_1–CO– represents a stearic acid residue (C_{18}) and R_4 a hydrogen atom; and echinocandin B, wherein R_1–CO– represents a linoleic acid residue (C_{18}, 2 double bonds) and R_4 a hydroxyl group.

[0020] Glycyrrhizin derivatives are known as components extracted from natural licorices and they are widely used for cosmetics and sweeteners. "Glycyrrhizin derivatives used according to the present invention" is a general term for compounds having a common basic framework represented by general formula (2):

[0021]

[Chemical Expression 7]



(2)

[0022] (in which formula: R_{11} to R_{15} are hydrogen atoms or appropriate substituents) and these may be single compounds or mixtures of compounds; to be specific, they may be exemplified by: 18α-glycyrrhitic acid [in the above-mentioned general formula (2), R_{11} denotes a glucuronylglucuronate residue; R_{12} a COOH group; R_{13} a CH_3 group; R_{14}, =O; and R_{15} a CH_3 group]; 18β-glycyrrhitic acid (the same groups as the foregoing); 18α-glycyrrhetinic acid [R_{11}, OH group; R_{12}, COOH group; R_{13}, CH_3 group; R_{14}, =O; and R_{15}, CH_3 group] 18β-glycyrrhetinic acid (the same groups as the foregoing); 3β-glucuronyl-18β-glycyrrhetinic acid [R_{11}, glucuronate residue; R_{12}, COOH group; R_{13}, CH_3 group; R_{14}, =O; and R_{15}, CH_3 group]; carbenoxolone [R_{11}, $COOCH_2CH_2COOH$ group; R_{12}, COOH group; R_{13}, CH_3 group; R_{14}, =O; and R_{15}, CH_3 group]; deoxoglycyrrhetinic acid [R_{11}, OH group; R_{12}, COOH group; R_{13}, CH_3 group; R_{14}, hydrogen atom; and R_{15}, CH_3 group]; 3α-dehydroxyglycyrrhetinic acid [R_{11}, =O; R_{12}, COOH group; R_{13}, CH_3 group; R_{14},=O; and R_{15}, CH_3 group]; hederagenin [R_{11}, OH group; R_{12}, CH_3 group; R_{13}, COOH group; R_{14}, hydrogen atoms; and R_{15}, CH_3 group]; 11-oxohederagenin [R_{11}, OH group; R_{12}, CH_3 group; R_{13}, COOH group; R_{14}, =O; and R_{15}, CH_2CH group]; oleanolic acid [R_{11}, OH group; R_{12}, CH_3 group; R_{13}, COOH group; R_{14}, hydrogen atom; and R_{15}, CH_3 group]; 11-oxo-oleanolic acid [R_{11}, OH group; R_{12}, CH_3 group; R_{13}, COOH group; R_{14}, =O; and R_{15}, CH_3 group]; and non-toxic salts thereof.

[0023] Salts of glycyrrhitic acid which have high solubilities are particularly preferred; non-toxic salts are exemplified by potassium, sodium, ammonium and hemisuccinate salts. Glycyrrhizin derivatives may be made into mono-, di- and tri- salts, depending on the numbers of their carboxylic acid groups: it is normally preferable to use 18β-glycyrrhitic acid, its dipotassium salt, its monoammonium salt, its diammonium salt, its disodium salt and its trisodium salt; and 18β-glycyrrhetinic acid and the disodium salt of carbenoxolone. The types of glycyrrhizin compounds according to the present invention include the above-mentioned free acids, salts and mixtures thereof.

[0024] The dosages to use of the aculeacins which are active ingredients according to the invention are made to be dosages whereby efficacy as pharmaceuticals (antimycotic effects and physiologically activating effects) will be expressed: in general, for adults, the dosages are 10 mg to 2 g per day. The compositional ratios of the aculeacins and glycyrrhizins will vary depending on the types and combinations of the aculeacins and glycyrrhizin derivatives: compositional ratios may be selected such that various aculeacins are solubilized. For example, when aculeacin Aγ is used, it is normally solubilized with approximately 1 to 4 parts by weight, or more, of dipotassium glycyrrhizate, or approximately 2 parts by weight, or more, of monoammonium glycyrrhizate, per 1 part by weight of the aculeacin. When

aculeacin Dγ is used, it is normally solubilized with approximately 1 part by weight, or more of dipotassium glycyrrhizate per 1 part by weight of the aculeacin.

[0025] The quantity of an aculeacin which is dissolved may be increased by the design of the process for solubilization. For example, when there is solubilization by using an ultrasonic cleaner the quantity of an aculeacin which dissolves is increased in proportion to the time used: when ultrasonic treatment is carried out for 60 seconds, there is solubilization with approximately 0.15 parts by weight, or more, of dipotassium glycyrrhizate or monoammonium glycyrrhizate per 1 part by weight of aculeacin Aγ.

[0026] The upper limit for the quantity of added glycyrrhizin derivatives is a quantity where the glycyrrhizin derivative itself will form a clear solution; this quantity will vary according to the type of glycyrrhizin derivative used, but, for dipotassium glycyrrhizate it is approximately 85 g/100 ml. The concentrations of glycyrrhizin derivatives in the solutions of the compositions according to the present invention may be appropriately selected: for example, the concentrations of glycyrrhizin derivatives which are the concentrations needed for the solubilization of aculeacins are approximately 0.001% to 30% of the concentrations of the

aculeacins, such as 0.001% to 85%, preferably 0.1% to 5%, more preferably 0.5 to 2%.

[0027] In order to manufacture compositions according to the present invention, the above-mentioned compositions of aculeacins and glycyrrhizin derivatives may, for example, be mixed together and, if required, there may also be appropriately admixed known pH regulators, isotonization agents, stabilizers, expanders, preservatives and the like. When mixing is carried out, this may be by mixing a solution, wherein a glycyrrhizin derivative is dissolved in an aforementioned aqueous solvent, and an aculeacin derivative, or by mixing an aqueous solvent, wherein is suspended an aculeacin, and a dry or dissolved glycyrrhizin derivative, or the dry constituent ingredients of each composition may be mixed directly in an aqueous solvent.

[0028] When solubilization is carried out, the means of doing so is not especially restricted, but it is particularly preferred to have ultrasonic treatment as previously described: the quantity dissolved is increased, depending on the length of time of the treatment. Treatment times from 5 to 120 minutes are preferred, more preferable times are from 30 to 60 minutes. When solutions of aqueous compositions and the like are manufactured, it is preferable to use sterile solvents, for example, sterile distilled water. It is also preferable to carry out a sterilization

treatment, such as by means of a 0.22 μm membrane filter, or another sterilization treatment by means of heat treatment, disinfectant gas, or the like.

[0029] The compositions according to the present invention may be either aqueous compositions or dry compositions thereof, but dry compositions are particularly preferred, because the aculeacins may thereby be stably stored. An example which may be given of a process to readily obtain dry compositions is to form dry material from a solution, once an aqueous composition has been made, by various means of drying. Examples of widely used means of drying are freeze drying, spray drying and reduced pressure drying, but freeze drying is particularly preferred.

[0030] To specifically exemplify an above-mentioned manufacturing process: manufacturing is carried out by solubilizing all of the components of an above-mentioned composition in sterile distilled water, after which sterile filtration is carried out by means of a 0.22 μm membrane filter and the filtrate is dispensed into vials, ampoules or the like, or freeze dried; aqueous compositions may be made when required for use by dissolution in distilled water for injection or the like. Many of the aqueous or dried compositions according to the present invention thus obtained may be used as injection agents, but they may also otherwise be used as formulations for oral or nasal administration.

[0031] The compositions according to the present inventtion are beneficial from the aspect of safety because glycyrrhizin derivatives are employed; these are very much safer than other solubilization agents which have been used; with regard to the stabilities of aculeacins in the compositions, excellent results for storage stabilities, particularly towards heat, are exhibited by the dry compositions. According to the present invention, the quantities of aculeacins dissolved are markedly increased by the addition of sodium salicylate or sodium benzoate as dissolution adjuvants, for increasing the solubilizing power for aculeacins.

[0032] When sodium salicylate or sodium benzoate are added, the quantities of aculeacins dissolved are increased in a dose-dependent manner by adding 0.1 or more parts by weight of sodium salicylate, or 2 or more parts by weight of sodium benzoate per 1 part by weight of the glycyrrhizin derivative. For example, the quantity of aculeacin Aγ dissolved when 0.2 parts by weight of sodium salicylate are added to 1 part by weight of dipotassium glycyrrhizate is approximately 6.5 parts by weight per 1 part by weight of dipotassium glycyrrhizate, but, when the quantity of sodium salicylate added is made 5 parts by weight, the quantity of the aculeacin dissolved is increased approximately 3-fold, up to 18 parts by weight.

[0033] Similarly, the quantity of aculeacin Aγ dissolved when 2 parts by weight of sodium benzoate are added to 1 part by weight of dipotassium glycyrrhizate is approximately 6 parts by weight per 1 part by weight of dipotassium glycyrrhizate, but, when the quantity of sodium benzoate added is made 10 parts by weight, the quantity of the aculeacin dissolved is increased up to 16 parts by weight. The above-mentioned aqueous or freeze-dried compositions containing aculeacins and glycyrrhizin derivatives, or aqueous compositions or redissolved freeze-dried compositions thereof, containing aculeacins and glycyrrhizin derivatives, and sodium salicylate or sodium benzoate are clear and they may be safely administered as pharmaceutical products.

[0034] When the above-mentioned aculeacin compositions are mixed with known infusions, if the quantity of the solubilizing agent is particularly small, turbidity is seen after mixing. The turbidity may be prevented by the addition of a specified amino acid, such as glutamic acid, aspartic acid or cysteine hydrochloride. For example, when 1 part by weight of aculeacin Aγ is solubilized with 2 parts by weight or less of dipotassium glycyrrhizate and diluted 50-fold with physiological saline, the solution is turbid. At this point, the turbidity may be prevented when 0.1 parts by weight, or more, of an amino acid, such as L-aspartic acid,

L-glutamic acid or cysteine hydrochloride, are added per 1 part by weight of potassium glycyrrhizate.

[0035] The above-mentioned amino acids may be added to compositions containing aculeacins and glycyrrhizin derivatives and they may be added to compositions containing aculeacins, glycyrrhizin derivatives and sodium salicylate or sodium benzoate. The quantities of the amino acids to add will vary depending on the types and quantities of the solubilizing agents used, but it is normally best to use quantities such that the content of amino acids is 0.1 to 0.5 parts by weight per 1 part by weight of solubilizing agent, or approximately 1% to 2% of an aqueous composition. There is no limitation on the order of addition of the amino acids and the above-mentioned sodium salicylate or sodium benzoate.

[0036]

[Examples] The present invention will be described below by giving Examples of its execution, without the present invention being limited by these Examples.

[0037]

[Example 1] Sterile distilled water (100 ml) was added to aculeacin Aγ (100 mg) and dipotassium glycyrrhizate [manufactured by Maruzen Kasei K. K. (Maruzen Chemicals); 1000 mg] and solubilized by treatment with ultrasound for 10 minutes

in an ultrasonic cleaner (Type 2200, 45 kHz, 80 W, manufactured by Branson), and a clear solution was obtained. Aseptic filtration was then carried out, using a 0.22 μm membrane filter, after which dispensation of 1 ml portions into vials and freeze drying were carried out; a freeze-dried formulation (Formulation A) of the type which is diluted at the time of use was obtained by replacing the air with nitrogen, capping and sealing the vials.

[0038] A freeze-dried formulation (Formulation B) was then obtained by treating aculeacin Aγ (100 mg) and sodium deoxycholate (manufactured by Sigma, 1000 mg) in the same way as below[TNS1]. Formulation A and Formulation B were kept at 50°C; at each point in time, 3 vials were taken out, the contents[TNS2] are each dissolved in water:acetonitrile (48:52, 1 ml)and the quantities of aculeacin were determined using high performance liquid chromatography (HPLC).

[0039] HPLC Conditions

Column: internal diameter, 4.6 × 150 mm

Packing: YMC AM-302 ODS S-5 120 Å (manufactured by the YMC Company)

Mobile Phase: water:acetonitrile (48:52)

Detection: UV 220 nm

Flow rate: 0.8 ml/min

Samples: mobile phase (1 ml) was added to a sample; after dissolution, 5 μl were injected.

Results:

[0040]

[Table 1]

Measurements of Aculeacin Aγ Contents

Formulation	Solubilizing Agent	Mean Percentage Residue from 3 Vials (%)		
		Start Time	1 Week at 50°C	2 Weeks at 50°C
A	Dipotassium glycyrrhizate	100	79.0	72.6
B	Sodium deoxycholate	100	31.5	30.0

[0041] As shown in Table 1, the aculeacin Aγ content (% residue) after storage at 50°C for 2 weeks was 72.6% for Lot A and 30.0% for Lot B: the heat storage stability of the formulation using dipotassium glycyrrhizate greatly surpassed that of the one formulated with sodium deoxycholate. Both samples formed clear solutions when water or water:acetonetrile were added.

[0042]

[Example 2] Dipotassium glycyrrhizate (400 mg) was added to aculeacin Aγ (100 mg); to these were added sterile distilled water (100 ml) and solubilization was carried out by stirring, after which the solution was aseptically filtered through a 0.22 μm membrane filter and 1 ml portions were

aseptically dispensed into ampoules and melt sealed, so that aqueous injection agents were obtained.

[0043]

[Example 3] Sterile distilled water (400 ml) was added to monoammonium glycyrrhizate (manufactured by Maruzen Kasei K. K.; (800 mg); the pH was adjusted to 7.0, using sodium hydroxide and hydrochloric acid, after which sterile distilled water was added to make the total volume 500 ml. To this was added aculeacin Aγ (100 mg) and solubilization was carried out by means of an ultrasonic cleaner (for 10 minutes), after which the solution was aseptically filtered through a 0.22 μm membrane filter, then 5 ml portions were aseptically dispensed into ampoules and melt sealed.

[0044]

[Example 4] Dipotassium glycyrrhizate (500 mg) was added to aculeacin Dγ (50 mg) and solubilization was carried out by adding sterile distilled water (50 ml) and using an ultrasonic cleaner. The solution was aseptically filtered through a 0.22 μm membrane filter, after which 1 ml portions were aseptically dispensed into vials, freeze drying was carried out, the vials were filled with nitrogen, capped and sealed, so that formulations for dissolution at the time of use were obtained.

[0045]

[Example 5] Sterile distilled water (100 ml) was added to aculeacin Aγ (100 mg), dipotassium glycyrrhizate (600 mg) and mannitol (400 mg), which were solubilized by stirring. The solution was aseptically filtered through a 0.22 μm membrane filter, after which 1 ml portions were aseptically dispensed into vials, freeze drying was carried out, so that injection agents for dissolution at the time of use were obtained.

[0046]

[Example 6] Sterile distilled water (100 ml) was added to aculeacin Aγ (100 mg), dipotassium glycyrrhizate (500 mg) and glucose (500 mg), which were solubilized by stirring. The solution was aseptically filtered through a 0.22 μm membrane filter, after which 1 ml portions were aseptically dispensed into vials. The whole contents of 1 of these vials were sucked out by means of a syringe and added to physiological saline for a drip infusion (500 ml), so that an injection agent for drip infusion was obtained.

[0047]

[Example 7]

Sterile distilled water (40 ml) was added to aculeacin Aα (40 mg) and dipotassium glycyrrhizate (160 mg), which were solubilized by stirring. The solution was filtered through a 0.22 μm membrane filter, after which 1 ml portions were aseptically dispensed into vials and freeze drying was

carried out, so that injection agents for dissolution at the time of use were obtained.

[0048]

[Example 8] Sterile distilled water (100 ml) was added to dipotassium glycyrrhizate (400 mg) and aculeacin Dα (40 mg), which were solubilized using an ultrasonic cleaner. The solution was aseptically filtered through a 0.22 μm membrane filter, after which 1 ml portions were aseptically dispensed into vials, freeze drying was carried out, the vials were filled with nitrogen, capped and sealed, so that injection agents for dissolution at the time of use were obtained.

[0049]

[Example 9] Sterile distilled water (20 ml) was added to aculeacin Aγ (500 mg) and dipotassium glycyrrhizate (200 mg) and solubilization was carried out using an ultrasonic cleaner (for 60 minutes). The solution was aseptically filtered through a 0.22 μm membrane filter, after which 0.5 ml portions were aseptically dispensed into vials and freeze drying was carried out, so that injection agents for dissolution at the time of use were obtained.

[0050]

[Example 10] Sterile distilled water (10 ml) was added to aculeacin Aγ (1500 mg), dipotassium glycyrrhizate (100 mg) and sodium salicylate (500 mg) and solubilization was

carried out using an ultrasonic cleaner (for 60 minutes). The solution was aseptically filtered through a 0.22 μm membrane filter, after which 0.5 ml portions were aseptically dispensed into vials and freeze drying was carried out, so that injection agents for dissolution at the time of use were obtained.

[0051]

[Example 11] Sterile distilled water (8 ml) was added to monoammonium glycyrrhizate (manufactured by Maruzen Kasei K. K.; 100 mg) and the pH was adjusted to 6.0, using sodium hydroxide and hydrochloric acid, after which the total volume was made 10 ml by adding sterile distilled water. To this was added aculeacin Aγ (1000 mg) and solubilization was carried out using an ultrasonic cleaner (for 60 minutes), after which the solution was aseptically filtered through a 0.22 μm membrane filter, then 0.5 ml portions were aseptically dispensed into vials and melt sealed.

[0052]

[Example 12] Sterile distilled water (10 ml) was added to aculeacin Aγ (500 mg), dipotassium glycyrrhizate (100 mg) and sodium benzoate (500 mg) and solubilization was carried out using an ultrasonic cleaner (for 60 minutes). The solution was aseptically filtered through a 0.22 μm membrane filter, after which 0.5 ml portions were aseptically dispensed into

vials and freeze drying was carried out, so that injection agents for dissolution at the time of use were obtained.

[0053]

[Example 13] Sterile distilled water (10 ml) was added to aculeacin Aγ (250 mg), dipotassium glycyrrhizate (100 mg) and aspartic acid (10 mg) and solubilization was carried out using an ultrasonic cleaner (for 60 minutes). The solution was filtered through a 0.22 μm membrane filter, after which 1 ml portions were aseptically dispensed into vials, and freeze dried, so that an injection agent for dissolution at the time of use was obtained. Sterile distilled water (1 ml) was added to 1 of these vials, so as to dissolve the contents and mixed with physiological saline solution (50 ml), so that a clear drip infusion agent was obtained.

[0054]

[Example 14] Sterile distilled water (10 ml) was added to aculeacin Aγ (230 mg), dipotassium glycyrrhizate (100 mg) and glutamic acid (10 mg) and solubilization was carried out using an ultrasonic cleaner (for 60 minutes). The solution was filtered through a 0.22 μm membrane filter, after which 1 ml portions were aseptically dispensed into vials and freeze dried, so that injection agents for dissolution at the time of use were obtained. Sterile distilled water (1 ml) was added to 1 of these vials, so as to dissolve the contents

and mixed with physiological saline solution (50 ml), so that clear drip infusion agents were obtained.

[0055]

[Example 15] Sterile distilled water (10 ml) was added to aculeacin Aγ (220 mg), dipotassium glycyrrhizate (100 mg) and cysteine hydrochloride (10 mg) and solubilization was carried out using an ultrasonic cleaner (for 60 minutes). The solution was filtered through a 0.22 μm membrane filter, after which 1 ml portions were aseptically dispensed into vials and freeze dried, so that injection agents for dissolution at the time of use were obtained. Sterile distilled water (1 ml) was added to 1 of these vials, so as to dissolve the contents and mixed with physiological saline solution (50 ml), so that clear drip infusion agents were obtained.

[0056]

[Effects of the Invention] It is possible to very safely provide aqueous compositions of aculeacins, or dried compositions thereof, by using glycyrrhizin derivatives as solubilization agents for aculeacins, which are sparingly soluble in water. The storage properties of the aculeacins in the compositions according to the present invention are excellent. The solubilities of aculeacins may be further increased by adding sodium salicylate or sodium benzoate to the above-mentioned compositions. It is possible to obtain

formulations without turbidity by further adding amino acids and mixing them into infusions.

Continued from Front Page

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(54)【発明の名称】　アクレアシン類の可溶化剤および医薬組成物

(57)【要約】

【構成】　本発明はアクレアシン類とグリチルリチン類とを含有することを特徴とするアクレアシン類水性組成物またはその乾燥組成物である。また、上記組成物にアクレアシン類の可溶化を高めるための溶解補助剤としてサリチル酸ナトリウムまたは安息香酸ナトリウムを添加してなる組成物である。さらにまた、本発明は、上記組成物を輸液に溶解したときに白濁を防止するために、上記組成物にアミノ酸を添加してなる組成物である。

【効果】　水に難溶性であるアクレアシン類の対して、可溶化剤としてグリチルリチン類を用いることにより、安全性が高く、可溶化された水性組成物またはその乾燥組成物を提供することが可能となった。

1

【特許請求の範囲】

【請求項1】　グリチルリチン類を有効成分とするアクレアシン類の可溶化剤。

【請求項2】　アクレアシン類とグリチルリチン類とを含有することを特徴とするアクレアシン類水性組成物またはその乾燥組成物。

【請求項3】　アクレアシン類とグリチルリチン類とサリチル酸ナトリウムまたは安息香酸ナトリウムとを含有することを特徴とするアクレアシン類水性組成物またはその乾燥組成物。

【請求項4】　アクレアシン類とグリチルリチン類とアミノ酸とを含有することを特徴とするアクレアシン類水性組成物またはその乾燥組成物。

【発明の詳細な説明】

【0001】

【産業上の利用分野】本発明は、アクレアシン類の可溶化剤及び医薬組成物に関する。

【0002】

【従来の技術】アクレアシン類は、アスペルギルス属に属する微生物により生産され、抗真菌作用を有する抗生物質として知られている（特公昭59－20350号公報、特公昭59－20351号公報、特公昭59－20352号公報、特公昭59－20353号公報）。またニューモシスチス・カリニ肺炎に対する予防及び治療薬としても期待されている〔特開平2－288837号公報、Tetrahedron Letters, 4147－4150（1976）, Helv. Chim. Acta., 62（4）, 1252－1267（1979）〕。

【0003】しかしながら、アクレアシン類は水に極めて難溶であり、水溶液中で均一に分散し、肉眼的に完全に澄明な状態になるまでに可溶化することは困難である。このため従来可溶化方法としてはアルコール、多価アルコール、コール酸類等の可溶化剤が用いられていた（特開平2－288837号公報）。これらの方法では、可溶化した後、生理食塩水等で希釈した場合、白濁することがあるので、さらに非イオン性界面活性剤、例えば、HCO－60、TWEEN－80などの添加が必要である。一方、非イオン性界面活性剤単独での可溶化は困難であった。さらに上記の界面活性剤などの溶解補助剤の添加では、安全性の面でも問題があった。

【0004】

【発明が解決しようとする課題】このようにアクレアシン類を可溶化するためには、アルコール、多価アルコール、コール酸類、非イオン界面活性剤等の使用が必須であったが、安全性の面で問題があった。従って、本発明は、安全で、安定なアクレアシン類組成物の提供を目的とする。

【0005】

【課題を解決するための手段】以上の問題点を解決すべく、可溶化方法について鋭意研究した結果、意外にもグリチルリチン類を可溶化剤として用いることにより、安全性が高く効率的にアクレアシン類を可溶化ができる組成物を得ることが可能となった。しかも、その組成物は保存安定性にも優れていることを見出した。

2

【0006】すなわち、本発明は、グリチルリチン類を有効成分とするアクレアシン類の可溶化剤を提供するものである。また、本発明は、アクレアシン類とグリチルリチン類とを含有することを特徴とするアクレアシン類水性組成物またはその乾燥組成物を提供するものである。本発明者は、さらに、アクレアシン類の可溶化力を高めるために溶解補助剤の検討を行ったところ、驚くべきことにサリチル酸ナトリウムまたは安息香酸ナトリウムを上記組成物に添加することにより、アクレアシン類の溶解量が著しく増量することを見出した。

【0007】すなわち、本発明は、アクレアシン類とグリチルリチン類とサリチル酸ナトリウムまたは安息香酸ナトリウムとを含有することを特徴とするアクレアシン類水性組成物またはその乾燥組成物を提供するものである。一般に、医薬品を投与する方法としては、静脈内、筋肉内、皮内および皮下投与などが考えられ、静脈内投与する場合、医薬品を輸液に混ぜた後、静脈内投与する方法が使用頻度の最も高い方法の一つであるが、本発明者は、さらに、上記アクレアシン類組成物の投与方法について検討を行ない、上記組成物を輸液に混ぜたところ、可溶化剤の含有量が著しく少量の場合は、混ぜた後に白濁が見られた。この白濁を防止するために、種々検討を加えた結果、上記組成物に特定のアミノ酸、例えばグルタミン酸、アスパラギン酸、システイン塩酸塩などを添加することにより白濁が防げることを見出した。

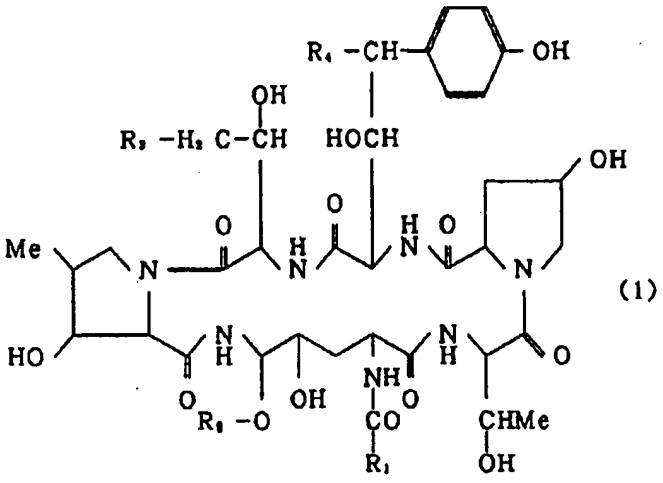
【0008】すなわち、本発明は、アクレアシン類とグリチルリチン類とアミノ酸とを含有することを特徴とするアクレアシン類水性組成物またはその乾燥組成物を提供するものである。ここで本発明にいう可溶化とは、アクレアシン類が水性溶媒中で均一に分散し、肉眼的に完全に澄明な状態になることをいう。この水性溶媒としては蒸留水が好適な例として挙げられる。さらにこれに適宜塩類、糖類や酸などが添加されていてもよく、これらの例として注射用蒸留水、生理食塩液、糖液、緩衝液等が挙げられる。さらに前記の水性溶媒は毒性を示さない限り水溶性有機溶媒、例えば少量のエタノール等を含んでいてもよい。本発明で水性組成物とは上記水性溶媒によりアクレアシン類が可溶化した溶液状の組成物を意味し、その水性組成物は適宜常法の乾燥手段により乾燥組成物として調製してもよい。

【0009】本発明の有効成分のアクレアシン類とは一般式（1）

【0010】

【化1】

3　　4



(1)

【００１１】（式中、R_1 －Ｏ－は長鎖飽和または不飽和脂肪酸残基またはベンゼン環、ピリジン環、酸素原子、イオウ原子または窒素原子を分子中に含有してもよい有機酸残基を示し、R_2 は水素原子、分鎖を有してもよい低級アルキル基、ベンジル基またはアミノ基がモノ低級アルキル基またはジ低級アルキル基で置換されてもよいアミノ－低級アルキル基を示し、R_3 は水素原子または－ＣＯＮＨ$_2$ 基を示し、R_4 は水素原子または水酸基を示す）で表される物質である。

【００１２】さらに、一般式（１）において、基R_1 の例としては、例えば、

【００１３】

【化２】

5 6

$-(CH_2)_n Me$ (n=10～20)、

$-(CH_2)_n CH=CH(CH_2)_7 Me$ (n=7, 9, 11)、

$-(CH_2)_8 CH=CH(CH_2)_7 Me$、

$-(CH_2)_7 CH=CH(CH_2)_8 Me$、

$-(CH_2)_4 CH=CH(CH_2)_{10} Me$、

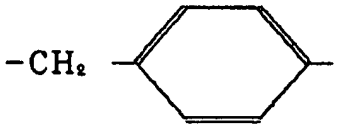
$-(CH_2)_7 CH=CHCH_2 CH=CH(CH_2)_4 Me$、

$-(CH_2)_7 CH=CHCH_2 CH=CHCH_2 CH=CHCH_2 Me$、

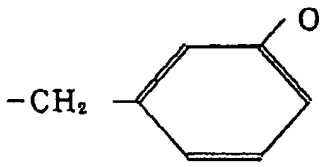
$-(CH_2)_8 CH(Me)-CH_2 CH(Me)-CH_2 Me$、

$-(CH_2)_{10}NHCO(CH_2)_n Me$ (n=7, 10)、

$-(CH_2)_4 NHCO(CH_2)_{10} Me$、

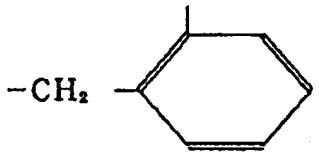


$-CH_2$ $O(CH_2)_n Me$ (n=7, 9, 11)、



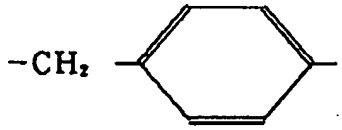
$O(CH_2)_n Me$ (n=7, 11)、

$-CH_2$

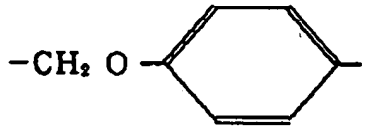


$O(CH_2)_7 Me$、

$-CH_2$



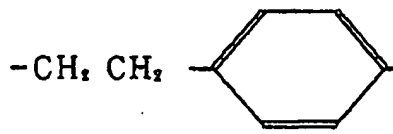
$-CH_2$ $NHCO(CH_2)_{10} Me$、

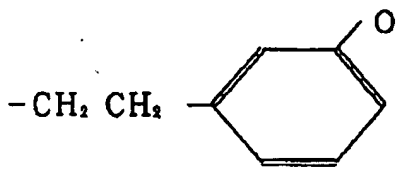


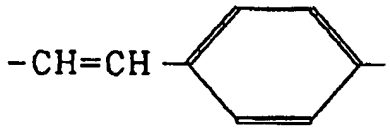
$-CH_2O$ $O(CH_2)_n Me$ (n=7, 9, 11)、

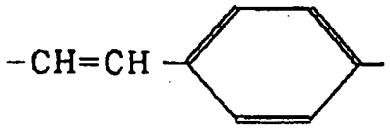
[0014] [化3]

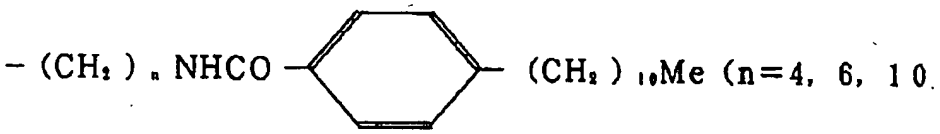
7 8

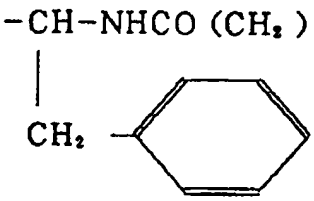

－CH₂ CH₂
O (CH₂) ₈ Me (n=3～8, 11)、

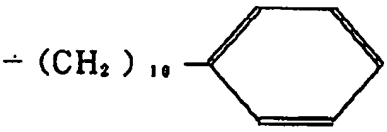

O (CH₂) ₈ Me、
－CH₂ CH₂

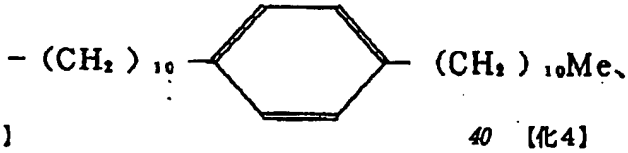

－CH=CH
O (CH₂) ₈ Me (n=5, 7, 9)、


－CH=CH
NHCO (CH₂) ₁₀Me、


－ (CH₂) ₄ NHCO
(CH₂) ₁₀Me (n=4, 6, 10


－CH－NHCO (CH₂) ₁₀Me、
CH₂

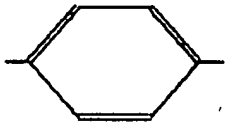

－ (CH₂) ₁₀

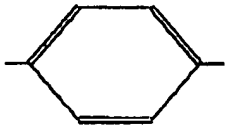

－ (CH₂) ₁₀
(CH₂) ₁₀Me、

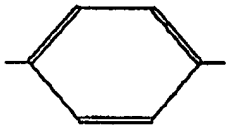
【0015】

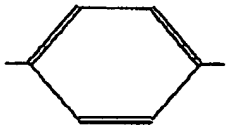
40 【化4】

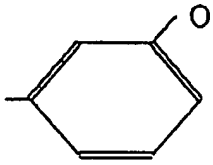
9 10

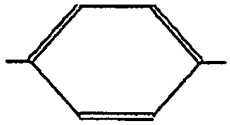

$(CH_2)_7Me$、

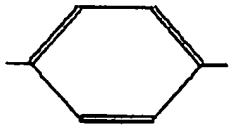

$O(CH_2)_nMe$ (n=5, 7, 9, 11, 13, 17)、

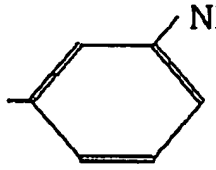

$S(CH_2)_nMe$ (n=7, 9, 11, 13)、


$SO_2(CH_2)_nMe$ (n=7, 9, 11, 13)、


$O(CH_2)_nMe$ (n=7, 9, 11, 13)、


NHCOMe、

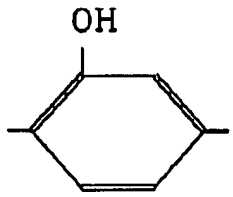

$NHCO(CH_2)_nMe$ (n=5, 8, 10, 12, 14)、

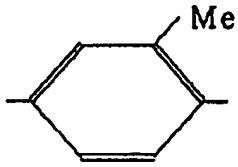

$NHCO(CH_2)_{10}Me$、

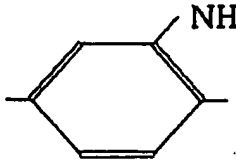
【0016】

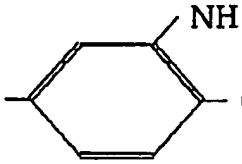
40 【化5】

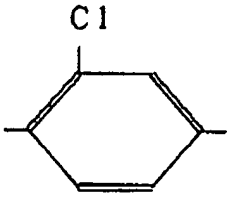
11　　12

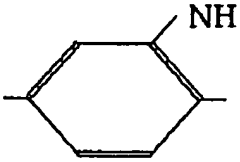

OH
NHCO (CH₂) ₁₀Me、

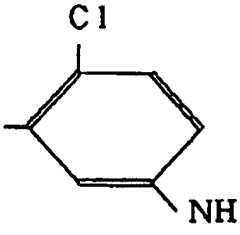

Me
NHCO (CH₂) ₁₀Me、


NHCO (CH₂) ₁₀Me、
Me

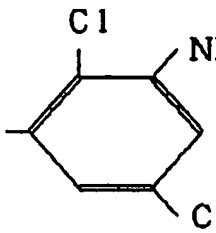

NHCO (CH₂) ₁₀Me、
OMe


Cl
NHCO (CH₂) ₙMe (n=6, 10)、

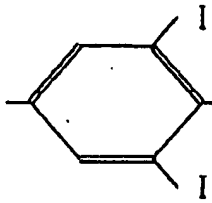

NHCO (CH₂) ₙMe (n=6, 10)、
Cl


Cl
NHCO (CH₂) ₙMe (n=6, 10)、

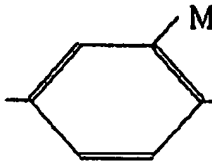
[0017]　　[化6]



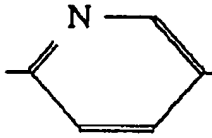
NHCO $(CH_2)_n$ Me (n=6, 8, 10, 12)、



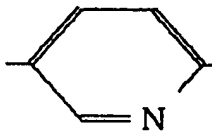
$NHCO(CH_2)_{10}Me$、



$CONH(CH_2)_{10}Me$、

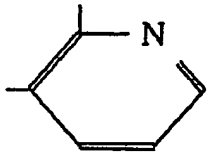


O $(CH_2)_n$ Me (n=7, 11)、



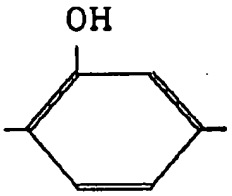
O $(CH_2)_n$ Me (n=7, 11)、

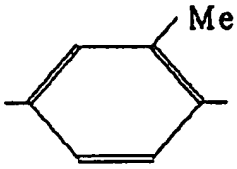
$NHCO(CH_2)_{10}Me$

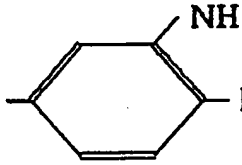


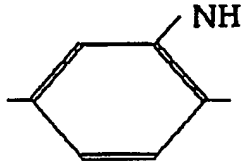
【0018】などが挙げられる。また、基R_3の例としては、例えば水素原子、メチル、エチル、プロピル、イソプロピル、ブチル、イソブチル、sec－ブチル、t－ブチル、ペンチル、ヘキシル、3－メチルブチル、2－エチルブチル、1－エチルブチルなどの直鎖または分鎖状の炭素数1～6の低級アルキル基、ベンジル基、2－アミノエチル、3－アミノプロピル、4－アミノブチル、2－アミノプロピル、2－アミノブチルなどのアミノ－低級アルキル基、アミノ基がメチル、エチル、プロピル、イソプロピル、ブチル、イソブチル、sec－ブチルなどのモノ低級アルキルまたはジ低級アルキル基で置換された2－アミノエチル、3－アミノプロピルなどのアミノ－低級アルキル基などが挙げられる。

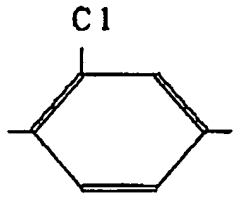
【0019】基R_3としては、水素原子または－$CONH_2$が挙げられ、基R_4としては水素原子または水酸基が挙げられる。上記一般式（1）において、R_1 －CO－が長鎖飽和または不飽和脂肪酸残基、例えば炭素数14～18（C_{14}～C_{18}）であり、R_3が水素原子、R_3が水素原子、R_4が水素原子または水酸基であるアクレアシン誘導体が好ましく、さらに好ましい例として、R_1 －CO－がミリスチン酸残基（C_{14}）、R_4が水酸基で示されるアクレアシンAα、R_1 －CO－がパルミチン酸残基（C_{16}）、R_4が水酸基で示されるアクレアシンAγ、R_1 －CO－がミリスチン酸残基、R_4が水素原子で示されるアクレアシンDα、R_1 －CO－がパルミチン酸残基、R_4が水素原子で示されるアクレアシンDγが挙げられ、さらにR_1 －CO－がステアリン酸残基（C_{18}）、R_4が水素原子で示されるエキノキャンディンC、R_1 －CO－がリノール酸残基（C_{18}，2重結合2個）、R_4が水酸基で示されるエキノキャンディン

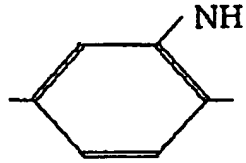

OH
NHCO (CH₂) ₁₀Me、

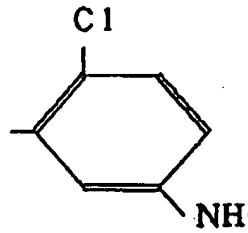

Me
NHCO (CH₂) ₁₀Me、


NHCO (CH₂) ₁₀Me、
Me


NHCO (CH₂) ₁₀Me、
OMe


Cl
NHCO (CH₂) ₙMe (n=6, 10)、


NHCO (CH₂) ₙMe (n=6, 10)、
Cl


Cl
NHCO (CH₂) ₙMe (n=6, 10)、

[0017]　　[化6]

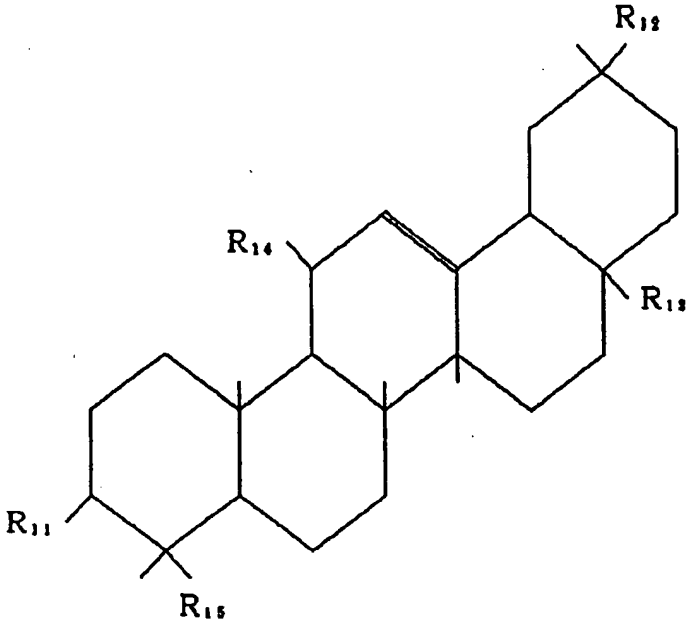
15　　16

Bなども好ましい例として挙げられる。

【0020】また、グリチルリチン類は、天然の甘草から抽出される成分として知られ、化粧品または甘味剤として幅広く用いられている。本発明で使用されるグリチ＊ルリチン類とは、一般式（2）

【0021】

【化7】



(2)

【0022】（式中、R_{11}～R_{15}は水素原子または適宜の置換基を示す）で表される基本骨格を共通とする化合物の総称であり、これらの単一の化合物またはこれらの化合物の混合物であってもよく、具体的には18α－グリチルリチン酸〔上記一般式（2）におけるR_{11}はグルクロニル－グルクロン酸残基、R_{12}はCOOH基、R_{13}はCH_3基、R_{14}は＝O基、R_{15}はCH_3基をそれぞれ示す〕、18β－グリチルリチン酸（同前）、18α－グリチルレチン酸（R_{11}；OH基、R_{12}；COOH基、R_{13}；CH_3基、R_{14}；＝O基、R_{15}；CH_3基）、18β－グリチルレチン酸（同前）、3β－グルクロニル－18β－グリチルレチン酸（R_{11}；グルクロン酸残基、R_{12}；COOH基、R_{13}；CH3基、R_{14}；＝O基、R_{15}；CH_3基）、カルベノキソロン（R_{11}；$COOCH_2CH_2COOH$基、R_{12}；COOH基、R_{13}；CH_3基、R_{14}；＝O基、R_{15}；CH_3基）、デオキソグリチルレチン酸（R_{11}；OH基、R_{12}；COOH基、R_{13}；CH_3基、R_{14}；水素原子、R_{15}；CH_3基）、3α－デヒドロキシグリチルレチン酸（R_{11}；＝O基、R_{12}；COOH基、R_{13}；CH_3基、R_{14}；＝O基、R_{15}；CH_3基）、ヘデラゲニン（R_{11}；OH基、R_{12}；CH_3基、R_{13}；COOH基、R_{14}；水素原子、R_{15}；CH_3基）、11－オキソヘデラゲニン（R_{11}；OH基、R_{12}；CH_3基、R_{13}；COOH基、R_{14}；＝O基、R_{15}；CH_3CH基）、オレアノール酸（R_{11}；OH基、R_{12}；CH_3基、R_{13}；COOH基、R_{14}；水素原子、R_{15}；CH_3基）、11－オキソオレアノール酸（R_{11}；OH基、R_{12}；CH_3基、R_{13}；COOH基、R_{14}；＝O基、R_{15}；CH_3基）およびそれらの非毒性塩などが例示される。

【0023】特に溶解性の高いグリチルリチン酸塩が好ましく、その非毒性塩としてはカリウム塩、ナトリウム塩、アンモニウム塩、ヘミサクシネートなどが例示される。グリチルリチン類のカルボン酸の数により、モノ、ジ、トリ塩などとすることができ、通常、18β－グリチルリチン酸、同ジカリウム塩、同モノアンモニウム塩、同ジアンモニウム塩、同ジナトリウム塩、同トリナトリウム塩、18β－グリチルレチン酸、カルベノキソロンジナトリウムなどが好ましく使用される。本発明のグリチルリチン類とは、上記の遊離酸、塩またはこれらの混合物を包含する。

【0024】本発明の有効成分のアクレアシン類の使用量は、医薬品として有効な作用（抗菌作用、生理活性作用）を発現する量とし、一般的には成人1日あたり10mg～2g程度である。アクレアシン類とグリチルリチン類の組成比は、アクレアシン類とグリチルリチン類の種類およびその組合せにより異なり、各々のアクレアシン類が可溶化する組成比を適宜選択すればよい。例えば、通常、アクレアシンAγを使用する場合、その1重量部に対して、グリチルリチン酸ジカリウム塩は約1～4重量部以上、グリチルリチン酸モノアンモニウム塩では約2重量部以上で可溶化する。また、例えばアクレアシンDγを使用する場合、その1重量部に対して通常グリチルリチン酸ジカリウム約1重量部以上で可溶化する。

【0025】アクレアシンの溶解量は可溶化の方法を工

夫することによりさらに増加する。例えば、超音波洗浄器を使用して可溶化すると、使用時間に比例してアクレアシン類の溶解量は増加し、６０分間超音波処理をした場合、アクレアシンＡγ１重量部に対してグリチルリチン酸ジカリウム塩またはグリチルリチン酸モノアンモニウム塩は約０．１５重量部以上で可溶化する。

【００２６】グリチルリチン類の添加量の上限はグリチルリチン類自体が澄明な溶液となる量であり、その量はグリチルリチン類の種類により異なるが、グリチルリチン酸ジカリウム塩では８５ｇ／１００ｍｌ程度である。本発明の組成物の溶液の濃度は、適宜選択し得るが、例えばアクレアシン類の濃度として０．００１～３０％程度、グリチルリチン類濃度はアクレアシン類が可溶化するのに必要な濃度であり、例えば０．００１～８５％、好ましくは０．１～５％、さらに好ましくは０．５～２％が挙げられる。

【００２７】本発明の組成物を製造するためには、例えば、上記組成のアクレアシン類とグリチルリチン類とを混合し、必要であれば、さらに公知のｐＨ調整剤、等張化剤、安定化剤、増量剤、防腐剤等を適宜混合してもよい。混合に際しては、例えば、グリチルリチン類を前記の水性溶媒にて溶解した溶液とアクレアシン類を混合するか、アクレアシン類を分散した水性溶媒と、グリチルリチン類の乾燥または溶液を混合するか、または、それぞれの組成物の構成成分の乾燥物を直接水性溶媒に混合してもよい。

【００２８】可溶化に際してその手段は特に限定されないが、前述したように超音波処理すると特に好ましく、処理時間の長さに依存して溶解量は増加する。５～１２０分間の処理時間が好ましいが、さらに好ましくは３０～６０分である。水性組成物等の溶液を調製するに際しては、滅菌状態の溶液、例えば滅菌蒸留水等を用いることが好ましい。また、０．２２μｍのメンブランフィルターなどによる無菌処理や、その他の加熱処理、殺菌ガス等による無菌処理を行うことが好ましい。

【００２９】本発明の組成物は水性組成物あるいはその乾燥組成物のいずれでもよいが、乾燥組成物は、アクレアシン類が安定に保存されることから特に好ましい。乾燥組成物を簡単に得るには、一度水性組成物とした溶液を各種の乾燥手段により乾燥物とする方法などが挙げられる。乾燥手段としては、例えば、凍結乾燥、スプレードライ法、減圧乾燥などが汎用されているが、特に凍結乾燥法が好ましい。

【００３０】以上の製造法を具体的に例示すると、上記の組成の各成分を滅菌蒸留水にて可溶化した後、０．２２μｍのメンブランフィルターにより無菌濾過し、バイアル、アンプルなどに分注するか、または凍結乾燥することにより調製し、必要により用時、注射用蒸留水などで溶解して水性組成物とすればよい。このようにして得られた本発明の水性または乾燥組成物は、注射剤として用いられることが多いが、その他経口や経鼻投与用の製剤として使用することもできる。

【００３１】本発明の組成物は安全性の高いグリチルリチン類を用いることにより、他の可溶化剤を用いる製剤よりも安全性の面で有利であり、さらに組成物中でのアクレアシン類の安定性は、乾燥状態で特に熱に対する保存安定性において優れた結果を示した。本発明においては、アクレアシン類の可溶化力を高めるために、溶解補助剤としてサリチル酸ナトリウムまたは安息香酸ナトリウムを上記組成物に添加することにより、アクレアシン類の溶解量が著しく増加する。

【００３２】サリチル酸ナトリウムまたは安息香酸ナトリウムを添加する場合は、グリチルリチン類１重量部に対してサリチル酸ナトリウムは０．１重量部以上、また安息香酸ナトリウムは２重量部以上添加することにより用量依存的にアクレアシン類の溶解量は増える。例えば、グリチルリチン酸ジカリウム１重量部に対して、サリチル酸ナトリウム０．２重量部添加したときのアクレアシンＡγの溶解量は、グリチルリチン酸ジカリウム１重量部に対して約６．５重量部であるが、サリチル酸ナトリウムの添加量を５重量部にすると、アクレアシンの溶解量は約３倍の１８重量部まで増加する。

【００３３】同様に、グリチルリチン酸ジカリウム１重量部に対して、安息香酸ナトリウム２重量部添加したときのアクレアシンＡγの溶解量は、グリチルリチン酸ジカリウム１重量部に対して約６重量部であるが、安息香酸ナトリウムの添加量を１０重量部にすると、アクレアシンの溶解量は１６重量部まで増加する。以上に述べたアクレアシン類とグリチルリチン類を含有する水性組成物またはその凍結乾燥組成物、あるいはアクレアシン類とグリチルリチン類とサリチル酸ナトリウムまたは安息香酸ナトリウムを含有する水性組成物またはその凍結乾燥組成物の再溶解物は澄明であり、医薬品として安全に投与できるものである。

【００３４】上記のアクレアシン類組成物を公知の輸液に混ぜたところ、可溶化剤の含有量が著しく少量の場合は、混ぜた後に白濁が見られる。この白濁を防止するために、特定のアミノ酸、例えばグルタミン酸、アスパラギン酸、システイン塩酸塩を添加することにより白濁が防止することができる。例えば、アクレアシンＡγ１重量部に対して、グリチルリチン酸ジカリウム２重量部以下で可溶化させ、生理食塩液で５０倍以上に希釈すると白濁する。このとき、Ｌ－アスパラギン酸、Ｌ－グルタミン酸、システイン塩酸塩などのアミノ酸を、グリチルリチン酸ジカリウム１重量部に対して０．１重量部以上添加すると白濁が防止できる。

【００３５】上記のアミノ酸類を、アクレアシン類とグリチルリチン類を含有する組成物に添加してもよいし、アクレアシン類とグリチルリチン類とサリチル酸ナトリウムまたは安息香酸ナトリウムを含有する組成物に添加

してもよい。アミノ酸類の添加量は、可溶化剤の種類およびその使用量により異なるが、通常は可溶化剤1重量部に対して0．1～0．5重量部あるいは水性組成物の約1～2％程度含有するように使用すればよい。アミノ酸類の添加と上記のサリチル酸ナトリウムまたは安息香酸ナトリウムの添加順序などの限定はない。

【0036】

【実施例】以下に実施例を挙げて本発明を説明するが、本発明はこれに限定されるものではない。

【0037】

【実施例1】アクレアシンAγ100mgとグリチルリチン酸ジカリウム（丸善化成社製）1000mgをとり、無菌蒸留水100mlを加え、超音波洗浄器（BRANSON社製 Type2200型 45KHz、80W）にて10分間超音波処理し、可溶化させて澄明な溶液を得た。次いで0．22μmのメンブランフィルターにより無菌濾過後、バイアルに1mlずつ分注し、凍結乾燥を行い、窒素置換、打栓、巻き締めし、用時溶解型凍結乾燥製剤（製剤A）を得た。

*【0038】次にアクレアシンAγ100mgとデオキシコール酸ナトリウム（シグマ社製）1000mgをとり、以下同様に処理し、凍結乾燥製剤（製剤B）を得た。製剤Aおよび製剤Bを50℃に保存し、各時点で3バイアルずつを取り出し、各々を水：アセトニトリル（48：52）1mlにて溶解して、高速液体クロマトグラフィー（HPLC）を用いて、アクレアシンAγ含量を測定した。

【0039】HPLC条件

カラム：内径4．6×150mm

充填剤：YMC AM－302 ODS S－5 120Å（YMC社製）

移動相：水：アセトニトリル（48：52）

検出：UV220nm

流速：0．8ml／min

試料：サンプルに移動相1mlを加え溶解後5μl注入

結果

【0040】

【表1】

アクレアシンAγ含量の測定

製剤	可溶化剤	3バイアルの平均残存率（%）		
		開始時	50℃1週間	50℃2週間
A	グリチルリチン酸ジカリウム	100	79．0	72．6
B	デオキシコール酸ナトリウム	100	31．5	30．0

【0041】表1に示したように、50℃2週間保存後のアクレアシンAγ含量（残存%）は、LotAが72．6%、LotBが30．0%であり、デオキシコール酸ナトリウムで製剤化したものに比べグルチルリチン酸ジカリウムを用いて製剤化した方がはるかに熱に対する保存安定性が勝っていた。なおいずれのサンプルも水または水：アセトニトリルの添加により澄明な溶液となった。

【0042】

【実施例2】アクレアシンAγ100mgにグリチルリチン酸ジカリウム400mgを添加し、これに無菌蒸留水100mlを加え、撹拌し可溶化した後、0．22μのメンブランフィルターで無菌濾過し、無菌的に1mlずつアンプルに分注・熔閉し、水性注射剤を得た。

【0043】

【実施例3】グリチルリチン酸モノアンモニウム（丸善化成社製）800mgに無菌蒸留水400mlを加え、水酸化ナトリウム及び塩酸を用いてpHを7．0に調製した後無菌蒸留水を加え全量を500mlとした。これにアクレアシンAγ100mgを添加し、超音波洗浄器（10分間）によって可溶化させた後0．22μmのメンブランフィルターにて無菌濾過後、無菌的に5mlずつアンプルに分注し熔閉した。

【0044】

【実施例4】アクレアシンDγ50mgにグリチルリチン酸ジカリウム500mgを添加し、無菌蒸留水50ml を加え超音波洗浄器を利用し可溶化した。0．22μmのメンブランフィルターにて無菌濾過後、無菌的にバイアルに1mlずつ分注し、凍結乾燥を行い窒素充填、打栓、巻き締めし、用時溶解型製剤を得た。

【0045】

【実施例5】アクレアシンAγ100mg、グリチルリ

チン酸ジカリウム６００mg、マンニット４００mgをとり、これに無菌蒸留水１００ｍｌを加えて撹拌し可溶化した。０．２２μmのメンブランフィルターにて無菌濾過後、無菌的に１ｍｌずつバイアルに分注し凍結乾燥を行い用時溶解注射剤を得た。

【００４６】

【実施例6】アクレアシンAγ１００mg、グリチルリチン酸ジカリウム５００mg、グルコース５００mgをとり、これに無菌蒸留水１００ｍｌを加えて撹拌し可溶化し、０．２２μmのメンブランフィルターにて無菌濾過後、無菌的に１ｍｌずつアンプルに分注した。そのうち１本をとり、注射筒で全量吸引し５００ｍｌの点滴用生理食塩液に加え、点滴用注射剤を得た。

【００４７】

【実施例7】アクレアシンAα４０mgとグリチルリチン酸ジカリウム１６０mgをとり、無菌蒸留水４０ｍｌを加えて撹拌し可溶化し、０．２２μmのメンブランフィルターにて無菌濾過後、無菌的に１ｍｌずつバイアルに分注し凍結乾燥を行い、用時溶解注射剤を得た。

【００４８】

【実施例8】アクレアシンDα４０mgとグリチルリチン酸ジカリウム４００mgをとり、無菌蒸留水１００ｍｌを加え、超音波洗浄器を用いて可溶化した。０．２２μmのメンブランフィルターにて無菌濾過後、無菌的にバイアルに１ｍｌずつ分注し、凍結乾燥を行い、窒素充填、打栓、巻締めし、用時溶解注射剤を得た。

【００４９】

【実施例9】アクレアシンAγ５００mgとグリチルリチン酸ジカリウム２００mgをとり、無菌蒸留水２０ｍｌを加え、超音波洗浄器（６０分）を用いて可溶化した。０．２２μmのメンブランフィルターにて無菌濾過後、無菌的にバイアルに０．５ｍｌずつ分注し、凍結乾燥を行い、用時溶解注射剤を得た。

【００５０】

【実施例１０】アクレアシンAγ１５００mg、グリチルリチン酸ジカリウム１００mg及びサリチル酸ナトリウム５００mgをとり、無菌蒸留水１０ｍｌを加え、超音波洗浄器（６０分）を用いて可溶化した。０．２２μmのメンブランフィルターにて無菌濾過後、無菌的にバイアルに０．５ｍｌずつ分注し、凍結乾燥を行い、用時溶解注射剤を得た。

【００５１】

【実施例１１】グリチルリチン酸モノアンモニウム（丸善化成社製）１００mgに無菌蒸留水８ｍｌを加え、水酸化ナトリウム及び塩酸を用いてｐＨを６．０に調製した後無菌蒸留水を加え全量を１０ｍｌとした。これにアクレアシンAγ１０００mgを添加し、超音波洗浄器（６０分間）によって可溶化させた後０．２２μmのメンブランフィルターにて無菌濾過後、無菌的に０．５ｍｌずつアンプルに分注し熔閉した。

【００５２】

【実施例１２】アクレアシンAγ５００mg、グリチルリチン酸ジカリウム１００mg及び安息香酸ナトリウム５００mgをとり、無菌蒸留水１０ｍｌを加え、超音波洗浄器（６０分）を用いて可溶化した。０．２２μmのメンブランフィルターにて無菌濾過後、無菌的にバイアルに０．５ｍｌずつ分注し、凍結乾燥を行い、用時溶解注射剤を得た。

【００５３】

【実施例１３】アクレアシンAγ２５０mg、グリチルリチン酸ジカリウム１００mg及びアスパラギン酸１０mgをとり、無菌蒸留水１０ｍｌを加え、超音波洗浄器（６０分）を用いて可溶化した。０．２２μmのメンブランフィルターにて無菌濾過後、無菌的にバイアルに１ｍｌずつ分注し、凍結乾燥を行い、用時溶解注射剤を得た。このバイアル１本をとり無菌蒸留水１ｍｌを加えて溶解し、これを生理食塩液５０ｍｌ中に混合し澄明な点滴用製剤を得た。

【００５４】

【実施例１４】アクレアシンAγ２３０mg、グリチルリチン酸ジカリウム１００mg及びグルタミン酸１０mgをとり、無菌蒸留水１０ｍｌを加え、超音波洗浄器（６０分）を用いて可溶化した。０．２２μmのメンブランフィルターにて無菌濾過後、無菌的にバイアルに１ｍｌずつ分注し、凍結乾燥を行い、用時溶解注射剤を得た。このバイアル１本をとり無菌蒸留水１ｍｌを加えて溶解し、これを生理食塩液５０ｍｌ中に混合し澄明な点滴用製剤を得た。

【００５５】

【実施例１５】アクレアシンAγ２２０mg、グリチルリチン酸ジカリウム１００mg及びシステイン塩酸塩１０mgをとり、無菌蒸留水１０ｍｌを加え、超音波洗浄器（６０分）を用いて可溶化した。０．２２μmのメンブランフィルターにて無菌濾過後、無菌的にバイアルに１ｍｌずつ分注し、凍結乾燥を行い、用時溶解注射剤を得た。このバイアル１本をとり無菌蒸留水１ｍｌを加えて溶解し、これを生理食塩液５０ｍｌ中に混合し澄明な点滴用製剤を得た。

【００５６】

【発明の効果】水難溶性であるアクレアシン類に対して、可溶化剤としてグリチルリチン類を用いることにより、安全性が高く可溶化された水性組成物またはその乾燥組成物を提供することが可能となった。さらに、本発明の組成物はアクレアシン類の保存安定性に優れている。また、上記組成物にサリチル酸ナトリウムまたは安息香酸ナトリウムを添加することにより、アクレアシン類の溶解量を増加させることが可能となった。さらに、アミノ酸類を添加することにより輸液に混注しても白濁しない製剤を得ることが可能となった。

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